To the Chairman of the Scientific Jury determined by order No. I-45/26.03.2025 of the Director of SAIM-BAS

REVIEW

By: Prof. Hristo Miladinov Najdenski, DSci, Member of BAS, The Stephan Angeloff Institute of Microbiology (SAIM), Bulgarian Academy of Sciences (BAS)

Subject: competition for the academic position "Associate Professor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences, scientific specialty Virology, announced in the State Gazette No. 11 of 07.02.2025 for the needs of the Department of Virology, Laboratory "Experimental Chem otherapy of Influenza" at SAIM-BAS.

I declare that there are no conditions for a conflict of interest between me and the candidate, Chief Assistant Lora Simeonova Simeonova, PhD within the meaning of paragraph 1, points 2a, 3, 4 and 5 of the the Additional Provisions of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB). The required documents provided to me for preparing a review have been prepared in accordance with the requirements of SAIM-BAS and submitted on time. Chief Assist. Lora Simeonova, PhD is the only candidate in the competition.

1. Biographical data about the candidate: education, qualification, professional development

Chief Assist. Lora Simeonova completed her higher education at Sofia University "St. Kliment Ohridski", receiving in 2003 the "Bachelor" degree in the specialty "Molecular Biology", and in 2005 the "Master" degree in the specialty "Virology". In

the same year, she began working as a specialist in the laboratory "Experimental Chemotherapy of Influenza" at the Department of "Virology" at the SAIM-BAS. In 2011, she successfully defended her dissertation on the topic "Study of the combined effect of rimantadine and oseltamivir against influenza virus A" and acquired the Educational and Scientific Degree (ESD) "Doctor". In the period from 2007 to 2015, she worked as an "Assistant Professor" in the lab. "Experimental Influenza Chemotherapy", Department of Virology at the SAIM-BAS, and since 2015 and currently she is a "Chief Assistant" in the same laboratory. Since 2012 she has been the head of the "Experimental Influenza Chemotherapy" research activity, Chief Assistant Simeonova has completed a number of courses and specializations in France, Italy, Germany, Korea, etc., which undoubtedly enrich her professional skills and competencies.

2. Fulfillment of the minimum requirements for holding the academic position

From the submitted documentation and prepared references, it is evident that the candidate fulfills the minimum national and additional requirements for the scientific activity of the SAIM_BAS for holding the academic position of "Associate professor". A review of the submitted references shows that for some of the indicators (groups D and E), the number of required points is even higher. It should be emphasized that the candidate is recognized among the Bulgarian and international scientific communities. The assessment of the impact in scientific circles is the high number of citations - a total of 148 according to Scopus, mainly in scientific journals referenced and indexed in world-renowned databases, with a high impact factor.

3. General characteristics of the submitted scientific papers

In this competition, Dr. Simeonova has presented a total of 20 scientific articles, of which 18 have been published in journals referenced in world-renowned databases (Web of Science and Scopus) and 2 in peer-reviewed scientific collections.

The articles have been published in prestigious international journals with a high impact factor, with 5 of them falling into Q1, 6 into Q2, 4 into Q3, etc.

The most numerous group is the group of articles characterizing new antiviral substances of natural and synthetic origin. These studies are particularly relevant, given the ever-increasing and spreading resistance to existing drugs, the observed toxicity and antiproliferative effect in host cells, especially when they are used in high doses or for a long period of time. The main viral models include representatives of the families *Orthomyxoviridae* (influenza viruses type A and B), *Coronaviridae* and *Herpesviridae* and to a lesser extent *Picornaviridae* and *Adenoviridae* (mainly for selective screening of compounds and/or products for virucidal activity according to Bulgarian State Standard (BSS).

Extensive in vitro screening studies have demonstrated good antiviral and virucidal effects of postbiotic products originating from fermentation processes and involving lactic acid bacteria against herpes viruses. Bioactive metabolites from various types of lactic acid bacteria significantly inhibit extracellular HSV-1 and to a lesser extent virions of the fish Koi Herpes virus (KHV). Blocking of viral adsorption to host cells and a protective effect on healthy MDBK (Madin–Darby bovine kidney cells) and CCB (carp brain line) cells against subsequent experimental viral infection have been demonstrated (No. 9, 12, 16). A review article (No. 15) has analyzed the knowledge on the effect of probiotic supplements on Koi herpes virus disease, as one of the main factors causing significant economic losses in wild and cultivated carp populations worldwide. Against the background of the lack of effective treatment for this disease and the limited use of vaccines in fish, probiotic supplements are one of the most perspective, promising and safe alternatives to drugs that provide health benefits for fish, such as improving their immune resistance and accelerated growth. A significant place in Dr. Simeonova's research is occupied by natural products of plant origin, which in recent decades have prevailed among the sources of new therapeutic agents. The focus of a group of studies is the antiviral effect of plant extracts from the species Tanacetum vulgare, Carlina acanthifolia and Rhus typhina in cell cultures against a panel of RNA- and DNA-

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containing viruses. It has been proven that the crude aqueous-ethanolic extract of the aerial parts of the medicinal plant *Tanacetum vulgare* L. possesses significant antiviral activity against HSV-1, due to inhibition of viral replication, blocking the entry of the virus - the absorption stage and direct virucidal effects on extracellular virions. It exhibits low cytotoxicity in Hep-2 and MDBK cells and moderate cytotoxicity in MDCK cells. The observed effect on influenza A H3N2 virus *in vitro* is weaker, which is likely a result of interference with cellular signaling pathways involved in the replication cycle (No. 8).

Extensive studies on the chemical composition, antioxidant activity, antibacterial, antiviral and acetylcholinesterase inhibitory properties of the essential oil from the roots of the plant *Carlina acanthifolia* All. revealed eight new compounds possessing strong radical-scavenging activity, antibacterial and antiviral activity against human poliovirus-1 (LSc-2ab) by inhibiting the adsorption of viral particles on HEp-2 cell lines (No. 11).

Another plant species that is intensively used in folk medicine and phytotherapy, and is the subject of intensive research, is *Rhus typhina* L. In vitro studies on the anticarcinogenic, antiviral and antioxidant effects of a crude methanol extract of the leaves of *Rhus typhina* L. showed that the plant extract has antiproliferative activity against two human cell lines (breast cancer). A moderate inhibitory effect on the replication of IAV/H3N2 was found, as well as an effect on herpes viruses and on the adsorption of HSV-1 and IAV/H3N2 strains. The leaf extract of *R. typhina* also shows strong free radical scavenging activity. Taken together, all these studies demonstrate the pharmacological potential of these plant extracts and open up new horizons for application in the prevention and therapy of a number of viral infections (No. 13).

Of high social importance are also studies on the rapid and adequate diagnosis of viral infections, especially during frequent influenza epidemics, where monitoring of sensitivity to licensed preparations is an essential component in the treatment of influenza in Bulgaria and around the world. For this purpose, classical and molecular techniques were used to assess the sensitivity of influenza virus strains (H1N1) and (H3N2), isolated in Bulgaria during the period 2004-2007, to neuraminidase inhibitors and M2 blockers. Phenotypic enzyme activity tests established full sensitivity of the isolates to the neuraminidase inhibitors oseltamivir and zanamivir. Sequence analysis demonstrated two mutations (S31N and V27T) in the transmembrane region of the M2 protein, responsible for resistance to adamantanes in strain A/Sofia/1250 (H3N2). These studies show that the two antiviral substances can be effectively used for the treatment of severe and moderate infection (No. 6). Modern molecular methods have also been used to establish the viral etiology of some types of cancer, such as squamous cell carcinomas (SCCs). By means of PCR amplification of the viral genes E6, E2, E1 and L1, the presence of viral DNA (HPV 6) was established in the studied samples. The results not only support the existing data in the literature on the papillomavirus-induced etiology of this type of neoplasms, but also show that more precise diagnostics of formations with a suspected viral etiology is also important for determining their malignancy and stage of development (No. 19).

In a series of collective studies under the leadership of Acad. Galabov, combined approaches for the prevention and/or therapy of influenza infection are sought, using a strategy for the application of an etiological viral inhibitor and substances modifying the biological response in the host. The scheme includes in parallel antioxidants and immunomodulatory preparations with proven mono-effects on oxidative stress. The results obtained show that the application of antiviral therapy simultaneously with increasing antioxidant protection in the host is a good approach for controlling the infection and reducing the severity of its course (No. 1, 2, 5). In a review article, the epigenetic regulation in the cell under the influence and pressure of constantly changing environmental factors that can affect the host's susceptibility to viral infections is very competently examined. However, additional studies are needed to outline new sensitive epigenetic mechanisms in viruses that would reveal new targets for therapy (No. 20).

In the context of the "One Health" concept and against the background of the acute need for effective antiviral therapeutics or the high level of resistance to

existing ones, possible therapeutic approaches have been studied, using various substances of natural origin, including encapsulated multicomponent plant extracts in liposomes, probiotic cultures with antimicrobial activity, anti-influenza substances with antioxidant properties such as blood geranium extract, ellagic acid, oseltamivir and isoprinosine in various model systems (No. 14, 17). Last but not least in their importance are the studies of Dr. Simeonova on the pathogenesis of viral infections, as a basis for increasing the effectiveness of antiviral therapy and a better understanding of the mechanisms of "virus-host" interaction (No. 3, 4, 7).

4. Assessment of pedagogical training and teaching activity

Dr. Simeonova works as a "Chief Assistant" in the Laboratory "Experimental Chemotherapy of Influenza" at the Department of Virology at the SAIM-BAS and has over 19 years of work experience. She leads practical classes for students from Sofia University "St. Kliment Ohridski", Faculty of Biology in Virology, program "Pharmacy" in Bulgarian, as well as exercises in Microbiology and Virology, specialty "Pharmacy" in English. She also lectures on the same discipline in English. Under her supervision, one bachelor's and one master's thesis were successfully defended at New Bulgarian University.

5. Significance of the project activity and contributions to science and practice

Dr. Simeonova participated as a leader and participant in the development of a total of 9 projects, of which one is international with researchers from the Pasteur Institute (Paris) and Sciensano (Belgium), 4 projects with the National Science Foundation at the Ministry of Education and Science of the Republic of Bulgaria (one of which she is the leader), 2 with the Medical University (Varna), one with Sofia University "St. Kliment Ohridski" and one project with Lumicode LtD.

As a result of the long-term and systematic laboratory studies on viral pathogenesis and antiviral therapy, important scientific and applied scientific contributions have been presented, which I fully support. They not only indicate novelties, based on specifically established results and conclusions, but are related to current and significant issues for the control, prevention and treatment of infectious diseases, the protection of animal and human health and improving the quality of life. The accumulated experience and knowledge are fundamental for future research and approaches to search for and apply new antiviral agents of natural or synthetic origin for the prevention and therapy of respiratory viral infections, for the creation of new pharmaceutical formulations, the use of 3D models and model systems for studying toxicity, antiviral capacity and modifications of innate immunity in order to limit viral infection and reduce the severity of its course, etc.

6. Critical remarks and recommendations

I have no critical remarks on the submitted scientific works. I know the candidate and his/her overall scientific development. My general impression is that Dr. Simeonova is an ambitious, purposeful, well-prepared and responsible researcher. Her professional competence and methodological equipment make her a sought-after and desired partner for joint scientific research, not only at the SWAIM-BAS, but also outside it. Taking into account her proven teaching experience, I believe that Dr. Simeonova is able not only to implement national and international scientific projects, but also to create a school of young researchers in the field of virology.

Conclusion

The entire scientific, teaching and expert activity of Dr. Simeonova is in current scientific areas related to the resolution of important issues for epidemiology and medicine. The presented data convincingly testify to the high competence and erudition of the candidate, contributing to increasing the research and teaching capacity of the SAIM-BAS. The acquired experience, the active and fruitful work in the above-mentioned research areas will undoubtedly contribute to their successful development in the future. Fully covering the minimum requirements of the LDASRB, as well as the required criteria of the SAIM-BAS, and considering all the abovementioned arguments and facts, I propose to the esteemed Scientific Jury to award Dr. Lora Simeonova Simeonova the academic position of "Associate Professor" in the scientific specialty of Virology for the needs of the Department of Virology, Laboratory "Experimental Chemotherapy of Influenza" at the SAIM-BAS.

10.06.2025 г., Sofia

Signature: